

growth of metastases and primary tumors in mice. O'Reilly et al., (*Cell* 79(2): 315-328, 1994) demonstrated that human angiostatin inhibited metastasis of Lewis lung carcinoma in SCID mice. The same group 5 (O'Reilly, M. S. et al., *Nat. Med. (N. Y.)* 2(6): 689-692, 1996) subsequently showed that human angiostatin inhibited the growth of the human tumors PC3 prostate carcinoma, clone A colon carcinoma, and MDA-MB breast carcinoma in SCID mice. Human angiostatin also 10 inhibited the growth of the mouse tumors Lewis lung carcinoma, T241 fibrosarcoma and M5076 reticulum cell carcinoma in C57Bl mice. Because these enzymatically-prepared angiostatins are not well characterized biochemically, the precise composition of the molecules 15 is not known.

Angiostatins of known composition can be prepared by means of recombinant DNA technology and expression in heterologous cell systems. Recombinant human angiostatin comprising Kringle domains one through four 20 (K1-4) has been produced in the yeast *Pichia pastoris* (Sim et al., *Cancer Res* 57: 1329-1334, 1997). The recombinant human protein inhibited growth of endothelial cells in vitro and inhibited metastasis of Lewis lung carcinoma in C57Bl mice. Recombinant murine 25 angiostatin (K1-4) has been produced in insect cells (Wu et al., *Biochem Biophys Res Comm* 236: 651-654, 1997). The recombinant mouse protein inhibited endothelial cell growth in vitro and growth of primary Lewis lung carcinoma *in vivo*. These experiments demonstrated that 30 the first four kringle domains are sufficient for angiostatin activity but did not determine which kringle domains are necessary.

Cao et al. (*J. Biol. Chem.* 271: 29461-29467, 1996), produced fragments of human plasminogen by proteolysis and by expression of recombinant proteins in *E. coli*. These authors showed that kringle one and to a lesser extent kringle four of plasminogen were responsible for the inhibition of endothelial cell growth in vitro. Specifically, kringles 1-4 and 1-3 inhibited at similar concentrations, while K1 alone inhibited endothelial cell growth at four-fold higher concentrations.

10 Kringles two and three inhibited to a lesser extent. More recently Cao et al. (*J Biol Chem* 272: 22924-22928, 1997), showed that recombinant mouse or human kringle five inhibited endothelial cell growth at lower concentrations than angiostatin (K1-4). These

15 experiments demonstrated in vitro angiostatin-like activity but did not address in vivo action against tumors and their metastases.

PCT publication WO 95/29242 discloses purification of a protein from blood and urine by HPLC that inhibits 20 proliferation of endothelial cells. The protein has a molecular weight between 38 kilodaltons and 45 kilodaltons and an amino acid sequence substantially similar to that of a murine plasminogen fragment beginning at amino acid number 79 of a murine 25 plasminogen molecule. PCT publication WO 96/41194, discloses compounds and methods for the diagnosis and monitoring of angiogenesis-dependent diseases. PCT publication WO 96/35774 discloses the structure of protein fragments, generally corresponding to kringle 30 structures occurring within angiostatin. It also discloses aggregate forms of angiostatin, which have endothelial cell inhibiting activity, and provides a

means for inhibiting angiogenesis of tumors and for treating angiogenic-mediated diseases.

"Endostatin" is a 20-kDa (184 amino acid) carboxy 5 fragment of collagen XVIII, is an angiogenesis inhibitor produced by a hemangioendothelioma (O'Reilly, M. S. et al., *Cell (Cambridge, Mass.)* 88(2): 277-285, 1997); and WO 97/15666). Endostatin specifically inhibits 10 endothelial proliferation and inhibits angiogenesis and tumor growth. Primary tumors treated with non-refolded suspensions of *E. coli*-derived endostatin regressed to 15 dormant microscopic lesions. Toxicity was not observed and immunohistochemical studies revealed a blockage of angiogenesis accompanied by high proliferation balanced by apoptosis in tumor cells.

"Interferon .alpha." (IFN.alpha.) is a family of highly homologous, species-specific proteins that possess complex antiviral, antineoplastic and immunomodulating 20 activities (Extensively reviewed in the monograph "Antineoplastic agents, interferon alfa", American Society of Hospital Pharmacists, Inc., 1996). Interferon .alpha. also has anti-proliferative, and antiangiogenic properties, and has specific effects on 25 cellular differentiation (Sreevalsan, in "Biologic Therapy of Cancer", pp. 347-364, (eds. V.T. DeVita Jr., S. Hellman, and S.A. Rosenberg), J.B. Lippincott Co., Philadelphia, PA, 1995).

Interferon .alpha. is effective against a variety 30 of cancers including hairy cell leukemia, chronic myelogenous leukemia, malignant melanoma, and Kaposi's sarcoma. The precise mechanism by which IFN.alpha.